The Synthesis of Phosphatidylethanolamine and Phosphatidylserine Containing Acetylenic or Cyclopropane Fatty Acids and the Activity of These Phosphatides in Blood Coagulation[1]

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ABSTRACT

Six different phosphatides were made by combination of phosphorus oxychloride and either *t*-butyloxycarbonylaminoethanol or the phthalimidomethyl ester of anisyloxycarbonyl-L-serine with three different diglycerides. The diglycerides were *rac*-1,2-distearoloylglycerol, *rac*-1-stearoloyl-2-stearoylglycerol, and *rac*-1,2-di(9,10-methyleneoctadecanoyl) glycerol. The phosphatides were freed of protective groups by methods that did not cause rupture of the cyclopropane rings. The resulting phosphatides were purified by chromatography and evaluated for their effects on blood coagulation in vitro after solubilization with sodium desoxycholate. The phosphatidyl(distearoloyl)serine and the phosphatidyl di(9,10-methyleneoctadecanoyl) serine were as active as beef brain phosphatidylserine in the antithromboplastin test and the Hicks-Pitney test. The phosphatidyl(stearoloyl, stearoyl)serine was slightly less active in the Hicks-Pitney test. The phosphatidylethanolamines accelerated coagulation in the Hicks-Pitney test. The cyclopropane phosphatidylethanolamine was also tested in a test of prothrombin conversion using all purified components and was found to be active. The high activity of the cyclopropane phosphatidylserine is specially important because of its resistance to autoxidation.

INTRODUCTION

Phosphatidylserine has been shown to be a potent anticoagulant in vivo (1,2) and in vitro (3,4). The anticoagulant activity of phosphatidylserine depends on its degree of microdispersion (solubilization) in aqueous media, water clear sols being most active (1,3,5). [The object of solubilization is to produce water clear solutions with particles of colloidal dimensions. The relation between solubilization and activity and the effect of agents like sodium desoxycholate is discussed in Reference 1.] Phosphatides with long chain saturated fatty acids like phosphatidyl(distearoyl)serine cannot be solubilized. [Phosphatidyl(dihexanoyl)serine can be solubilized (6) but it has little activity in blood clotting tests (unpublished work of this laboratory). Phosphatidyl(dihexanoyl)ethanolamine can also be solubilized (7) but its activity if any is unrecorded.] The active phosphatides that can be solubilized ordinarily contain double bonds. These can be solubilized with the aid of sodium desoxycholate or albumin (3).

Unfortunately, the unsaturated phosphatides are subject to easy oxidation and this is particularly true of the more potent preparations like phosphatidyl(dilinoleoyl)serine (4). Phosphatidylserine is an anticoagulant with potential therapeutic activity, but there is no record of any attempt to develop it for this purpose. This may be partly due to the difficulty in standardization and storage which autoxidation would cause.

Phosphatidylethanolamine is also of great interest because of its demonstrated procoagulant activity (3) and its capacity to shorten the secondary bleeding time in hemophilic dogs (unpublished work of this laboratory).

Our interest in the acetylenic fatty acids as possible components of synthetic phosphatides arose from the statement of Meade (8) that "oxygen attacks simple acetylenes far less readily than the corresponding olefins presumably because the methylenic attack which leads to the symmetrical low energy intermediate -CH-CH-CH- has no equally powerful driving force in acetylenes." This statement is supported by the experimental work of Kuhn

[1]Presented at the AOCS Meeting, Minneapolis, October 1969.

TABLE I

Analysis of Products

Substance	Carbon		Hydrogen		Nitrogen		Phosphorus	
	Theory	Found	Theory	Found	Theory	Found	Theory	Found
Rac-stearoyl,stearoloyl PE	66.10	65.99	10.69	10.28	1.87	1.94	4.15	4.22
Rac-distearoloyl PE	66.46	66.17	10.20	10.55	1.89	1.95	4.18	4.21
Rac-stearoyl,stearoloyl PS	64.01	63.97	9.97	10.02	1.77	1.82	3.93	3.95
Rac-distearoloyl PS	64.34	64.66	9.51	9.60	1.76	1.85	3.95	4.24
Rac-di(9,10-methylene-octadecanoyl) PE	66.89	66.56	10.71	10.47	1.82	1.88	4.01	4.03
Rac-di(9,10-methylene-octadecanoyl) PS	64.75	64.71	10.13	10.10	1.72	1.69	3.79	3.79

and Meyer (9) who measured the oxygen uptake of solutions of oleic acid and stearolic acid in the presence of hemin when shaken with aqueous buffer and oxygen at 37 C. They found no oxidation of stearolic acid (Table II in Ref. 9; also 10). Khan et al. (11), on the other hand, found that methyl stearolate absorbed more oxygen than methyl oleate. In these experiments, oxygen was bubbled through at 75 C. This apparent contradiction will have to be resolved by further experiments.

According to the literature on dihydrosterculic acid and lactobacillic acid (12) the cyclopropane ring resists the attack of powerful oxidizing agents like potassium permanganate, monoperphthalic acid (12) and chromic acid (13). We decided to synthesize phosphatidylserine and phosphatidylethanolamine containing acetylenic and cyclopropane fatty acids to find out if these phosphatides could be solubilized and tested in blood coagulation systems.

The cyclopropane phosphatides might also be of interest for the study of bacterial metabolism since it has been shown by Zalkin et al. (14,15) that phosphatidylethanolamine is a precursor in the formation of cyclopropane fatty acids.

EXPERIMENTAL PROCEDURES AND RESULTS

Materials and Methods

Stearolic Acid. This was purchased from Farchan Research Laboratories, Willoughby, Ohio. Two samples were used. The first sample assayed 99.4% C_{18} monoyne by gas liquid chromatography (GLC). This was used to prepare *rac*-phosphatidyl(stearoloyl, stearoyl) ethanolamine and *rac*-phosphatidyl(stearoloyl, stearoyl)serine. The distearoloyl phosphatides were made from a second sample of stearolic acid which contained 95.2% C_{18} monoyne, 3.6% of C_{18} monoene and 1.2% of C_{18} diene. A small quantity of *rac*-phosphatidyl(distearoloyl)serine was also made from the first (99.4%) sample of stearolic acid. This material did not differ from the other phosphatidylserine (from the 95% sample) in dispersibility or effect in blood coagulation.

Stearic acid (99.5% pure) was purchased from Stearinerie Dubois Fils, Scoury, Commune de Ciron (Indre), France.

Cis-dl-9,10-Methyleneoctadecanoic Acid. This was made by the Simmons-Smith reaction (16,17) as modified by Christie and Holman (18). Christie and Holman describe the synthesis on a very small scale. For 20 g of methyl oleate, we employed 50 g of zinc dust converted to the copper couple with 200 g of diiodomethane in 400 ml of anhydrous ether. The product was freed of diiodomethane and purified on a florisil column as described (18). The product contained 87.4% of cyclopropane fatty ester as determined by GLC. The ester was saponified with alkali and the free acid was converted to the acid chloride with oxalyl chloride. The free acid had the infrared spectrum indicated in the literature (19).

t-Butyloxycarbonylaminoethanol was made as described by Daemen et al. (20). The phthalimidomethyl ester of *p*-methoxybenzyloxycarbonyl-L-serine was made as described (4).

Silicic acid was either Bio-Sil from Bio-Rad Laboratories, Richmond, Calif., or silicic acid type CC-7 from Mallinckrodt Chemical Works, St. Louis, Mo. In each case it was washed thoroughly with solvent as described by Rouser et al. (21). Silica Gel H for thin layer chromatography (TLC) was a product of Merck of Darmstadt, furnished by Brinkmann Instruments, Inc., Westbury, N.Y. DEAE cellulose was Whatman No. DE-23 from Reeve Angel, Inc., Clifton, N.J.

All operations were conducted under nitrogen, using the glove box of I2R Inc., Cheltenham, Pa.

TABLE II

GLC Analysis of Fatty Acid Content

Substance	C_{18} monoyne	C_{18} satd.	C_{18} monoene (?)[a]	C_{18} diene (?)[a]	C_{19} cyclopropane
Rac-stearoyl, stearoloyl PE	49.3%	50.7%	---	---	
Rac-distearoloyl PE	95.2%		3.6%		
Rac-stearoyl stearoloyl PS	49.1%	50.9%	---	---	
Rac-distearoloyl PS					
Sample 1	92.8%		5.8%	1.1%	
Sample 2	99.8%				
Rac-cyclopropane PE					91.1%
Rac-cyclopropane PS					93.3%

[a]The question mark indicates that the retention times were not quite identical to those of standard esters.

Carbon, hydrogen, nitrogen and phosphorus analyses were by Galbraith Laboratories, Inc., Knoxville, Tenn.

GLC Analyses. The analyses were made with an Aerograph 1522 (Varian Aerograph, Walnut Creek, Calif.) chromatograph equipped with dual hydrogen flame ionization detectors. The column was an 8 ft x 1/8 in. o.d. (0.093 in i.d.) stainless steel coiled tube packed with 12% stabilized diethylene glycol succinate (DEGS) on 60-80 mesh acid washed Chromosorb W and maintained at 175 C. The areas under the peaks of the individual components were determined by an electronic integrator (Infotronics, Model CRS-11HSB, Houston, Texas) coupled to a digital printer. The methyl esters of the fatty acids and phospholipids were prepared by the method of Luddy et al. (22). Identifications were made from comparison with reference samples when available and from published data on retention volumes.

Rac-1,2-Distearoloylglycerol. *Rac*-1-(2'-tetrahydropyranyl)-glycerol was made according to Barry and Craig (23). Stearoloyl chloride was made from stearolic acid and oxalyl chloride. Acylation of the glycerol derivative with the stearoloyl chloride was performed by the method used by Baer and Buchnea for the acylation of other glycerol derivatives (24). The tetrahydropyranyl protecting group was removed from the product by the method of Paltauf and Spener (25) to give the *rac*-1,2-distearoloylglycerol. This product was purified on a column of silicic acid by elution with *n*-hexane followed by 5% ether in hexane and 30% ether in hexane. The fractions were examined by TLC using a boric acid impregnated Silica Gel H (26) and the solvent *n*-hexane-ether-acetic acid 7:20:7 by comparison with a standard mixture of triglyceride, 1,2,- and 1,3-diglycerides and monoglyceride furnished by Applied Sciences, Inc., State College, Pa. The fraction eluted from the column with 30% ether in hexane was found to be the 1,2-diglyceride and showed only a single spot on GLC. This fraction was used for the synthesis of phosphatides.

Rac-1-Stearoloyl-2-Stearoylglycerol. 2-Stearoylglycerol was made according to Bogoslovskii et al. (27) from 1,3-benzylideneglycerol which was made according to Johary and Owen (28). To a solution of 15.0 g of 2-stearoylglycerol in 6.9 ml of pyridine and 200 ml of benzene stirred at 30 C was added dropwise in the course of 40 min, a solution of 10 g of stearoloyl chloride in 100 ml of benzene. The mixture was stirred for two days at room temperature. It was then diluted with anhydrous ether and centrifuged to remove pyridine hydrochloride. The ether-benzene was washed four times with water and dried over sodium sulfate. Evaporation gave an oil which was dissolved in 185 ml of hexane and cooled in ice water to give some recovered 2-stearoylglycerol. The filtrate from this was evaporated to an oil which was taken up in 175 ml of 95% ethanol. After stirring for 10 min at 30 C the mixture was centrifuged giving a trace of undissolved oil which was discarded.

The ethanol solution was evaporated to give a crystalline residue, mp 34-36 C. TLC of this material on silica gel impregnated with boric acid, using the solvent *n*-hexane-ether-acetic acid 70:20:7 showed only one spot which ran opposite authentic 1,2-diglyceride.

Analysis Calculated for $C_{39}H_{72}O_5$ (620.97): C, 75.42; H, 11.69. Found: C, 75.67; H, 11.75.

Rac-1,2-di(cis-dl-9,10-Methyleneoctadecanoyl)Glycerol. This was made using the cyclopropane fatty acid chloride as described above for the distearoloylglycerol. The purifi-

TABLE III

Procoagulant Activity of Solubilized[a] Phosphatidyl(Distearoloyl)Ethanolamine and Phosphatidyl(Stearoyl,Stearoloyl)Ethanolamine in the Modified Hicks-Pitney Test (3)

Substance tested	Micrograms in incubation mixture	Incubation time in minutes			
		2	4	6	8
		Substrate clotting time in seconds			
Distearoloyl PE	100	>50	17.0	11.2	8.8
	5	45.5	13.8	9.0	8.0
Stearoyl,stearoloyl PE	100	>80	36.5	30.0	15.8
	5	59.8	31.2	20.5	15.0
Controls					
Crude phosphatides (40)	6	85.0	12.0	7.8	7.8
Buffered saline (3)	---	>90	46.7	30.5	22.8
Sodium desoxycholate (3)	100	>90	68.0	33.0	23.0

[a]Each substance tested was solubilized in a solution of sodium desoxycholate in buffered saline (3).

cation was also similar. The product showed only one spot on TLC running with the same R_f as a standard 1,2-diglyceride.

Synthesis of Protected Phosphatides. The combination of the diglycerides with phosphorus oxychloride and the phthalimidomethyl ester of anisyloxycarbonyl-L-serine or *t*-butyloxycarbonylamino ethanol was conducted as described by Baer and Buchnea (29) except that the product was worked up in chloroform instead of ether (30,31).

Removal of the Phthalimidomethyl Group. This was done with 95% hydrazine dissolved in ethanol by incubation at 37 C for two days as described (31).

Removal of BOC and Anisyloxycarbonyl Protecting Groups. The product from the phosphorus oxychloride synthesis (in the case of phosphatidylethanolamine) or from the removal of the phthalimidomethyl group (in the case of phosphatidylserine) was dissolved in 97% formic acid and the solution was kept at room temperature for 3 hr (32). The solution was then freeze-dried to remove formic acid.

The phosphatidylserines were purified on a column of DEAE cellulose acetate by the method of Rouser et al. (21,33,34), the desired fraction being eluted with glacial acetic acid. The acetic acid was removed by freeze-drying. The product was examined by TLC using Silica Gel H and the solvent system of Grisdale and Okany (35), staining with ninhydrin and the phosphorus stain of Long et al. (36). The phosphatidylserines ran with the same R_f as a standard preparation of phosphatidylserine from beef brain. However, they contained a trace of impurity at the origin. To remove this impurity, the phosphatidylserines were chromatographed on silicic acid as described previously (4,31). The analyses of the products are shown in Table I and II.

The phosphatidylethanolamines were eluted from a DEAE cellulose acetate column with 10% methanol in chloroform and this fraction

TABLE IV

Procoagulant Activity of Solubilized[a] Phosphatidylethanolamine Containing Cyclopropane Fatty Acids in the Modified Hicks-Pitney Test (3)

Substance tested	Micrograms in incubation mixture	Incubation time in minutes			
		2	4	6	8
		Substrate clotting time in seconds			
Cyclopropane PE	200	85.3	35.2	24.0	7.5
	100	>90	35.0	9.5	9.0
	50	>90	60.5	48.0	43.0
Controls					
Crude phosphatides (40)	6	27.2	8.5	7.8	7.5
Buffered saline (3)	---	>90	89.6	44.0	37.0
Sodium desoxycholate (3)	200	>90	>90	>90	57.6

[a]Each substance was solubilized in a solution of sodium desoxycholate in buffered saline (3).

TABLE V

Anticoagulant Activities of Solubilized[a] Phosphatidyl(Distearoloyl)Serine, Phosphatidyl (Stearoloyl,Stearoyl)Serine and Beef Brain Phosphatidylserine in the Modified Hicks-Pitney Test (3) Versus Coagulant Phosphatides

Substance tested	Micrograms in incubation mixture	Incubation time in minutes			
		2	4	6	8
		Substrate clotting time in seconds			
Distearoloyl PS	10.0	>90	>90	>90	>90
	5.0	>90	71.8	82.2	38.0
	1.0	59.0	14.0	8.0	7.8
Stearoyl,stearoloyl PS	12.5	>90	>90	>90	>90
	10.0	>90	88.2	44.2	42.2
	5.0	>90	41.5	38.0	13.0
	1.0	>90	18.5	10.8	9.2
Beef brain PS (3)	10.0	>90	>90	>90	>90
	5.0	>90	81.0	50.0	52.5
	1.0	>90	31.0	24.2	10.8
Controls					
Crude phosphatides (40)	6	>90	19.0	8.8	8.5
Crude phosphatides + Desoxycholate	6 10	50.0	13.5	7.8	7.8
Buffered saline	---	>90	43.5	24.0	20.0

[a]Each substance tested was solubilized in a solution of sodium desoxycholate in buffered saline. All substances were tested for their anticoagulant activity against the acceleratory activity of crude phosphatides.

was further purified on Mallinckrodt CC-7 silicic acid. The pure material was eluted with 10% methanol in chloroform. The analyses of the phosphatidylethanolamines are shown in Tables I and II. The yield of pure phosphatide in these syntheses was about 500 mg from 5 g of diglyceride.

All of the six synthesized phosphatides were pure when compared to standard preparations by chromatography on Whatman SG-81 silica impregnated paper by the method of Marinetti (37). A load of 250 μg was employed.

Hydrolysis of Mixed-Acid Phosphatide. This was done using Russell's viper venom as described by DeHaas and Van Deenen (38) on a sample of *rac*-phosphatidyl(stearoloyl, stearoyl) ethanolamine using an equimolar amount of palmitic acid as a standard. TLC showed the production of equal amount of lysophosphatide and unchanged phosphatide as expected from the hydrolysis of a racemic phosphatide. GLC of the fatty acid methyl esters showed equal amounts of palmitic and stearic acids.

Procoagulant Activity of the Synthetic Phosphatidylethanolamines

The *rac*-phosphatidyl(distearoloyl)ethanolamine was shown to be a potent accelerator of thromboplastin generation. As little as 5 μg produced substrate clotting times of 9 sec in the test system shown in Table III. The *rac*-phosphatidyl(stearoloyl, stearoyl)ethanolamine was considerably less active. The solubilized phosphatidylethanolamine containing cyclopropane fatty acids was active in tests of thromboplastin generation at 100 μg in the incubation mixture (Table IV). At lower concentrations of the phosphatidylethanolamine the procoagulant activity was not evident. The phospholipid was also effective in the conversion of prothrombin to thrombin in a test system described elsewhere (39) which employs all purified clotting factors. The cyclopropane phosphatidylethanolamine (100 μg) was able to produce 5.1 units of thrombin after 1 min incubation, whereas crude phosphatides (40) produced 4.5 units. At lower concentrations of phosphatidylethanolamine the prothrombin converting activity fell off sharply.

Anticoagulant Activity of the Phosphatidylserines

All the synthetic phosphatidylserines were tested for their ability to interfere with thromboplastin generation and for their ability to overcome the strong procoagulant effect of brain thromboplastin. In addition, the cyclopropane phosphatide was tested for its ability to inhibit prothrombin conversion in a test system employing all purified components (39). In all cases there was a potent anticoagulant effect. In the prothrombin conversion test there was marked inhibition of rate and amount of

TABLE VI

Anticoagulant Activity of Solubilized Cyclopropane Phosphatidylserine Compared to That of Beef Brain Phosphatidylserine in the Modified Hicks-Pitney Test (3) Versus Coagulant Phosphatides (40)

Substance tested	Micrograms in incubation mixture	Incubation time in minutes			
		2	4	6	8
		Substrate clotting time in seconds			
Cyclopropane PS	10	>90	>90	>90	71.2
	7.5	>90	>90	>90	62.9
Beef brain PS	20	>90	>90	>90	98.0
	10	>90	69.5	46.5	12.8
Controls					
Crude phosphatides (40)	6	32.5	7.5	7.5	7.5
Buffered saline (3)	---	90	67.8	43.5	40.0
Sodium desoxycholate (3)	20	90	90	60.2	41.4

[a]Each substance was solubilized in a solution of sodium desoxycholate in buffered saline (3).

prothrombin conversion. With 10 μg of the cyclopropane phosphatide in the test system, 0.88 units of thrombin were obtained after 1 min of incubation of the prothrombin converter (plus inhibitor) and prothrombin, while the control (without cyclopropane PS) produced 2.0 units of thrombin. After 10 min of incubation, the control produced 6.24 units of thrombin while 10 μg of added cyclopropane PS reduced this to 3.68 units of thrombin. The anticoagulant effect against thromboplastin generation and against brain thromboplastin are shown in Tables V and VI.

In the Hicks-Pitney test the action of phosphatidylserine against the strong acceleratory activity of a crude thromboplastin fraction from brain tissue was examined. The strong anticoagulant activity of 10 μg of *rac*-phosphatidyl(distearoloyl)serine (Table V) or of *rac*-phosphatidyl di(9,10-methylene-octadecanoyl) serine (Table VI) is clear at 10 μg where the activity is similar to that of phosphatidylserine from beef brain. At lower concentrations the activity diminished, and with only 1 μg of phosphatidylserine in the test the substrate clotting times were similar to that of the control containing only the acceleratory crude phosphatides (Table V).

Table VII shows that the cyclopropane phosphatidylserine is equivalent to beef brain phos-

TABLE VII

Anticoagulant Activity of Solubilized[a] Cyclopropane Phosphatidylserine Compared to That of Beef Brain Phosphatidylserine in the Antithromboplastin and Recalcification (3) Tests

Substance tested	Microgram in clotting test	Antithromboplastin test; clotting time, sec	Recalcification test; clotting time, sec
Cyclopropane PS	200	176	2,750
	100	66	985
	50	48	485
	10	25	300
	1	14	210
Beef brain PS	200	173	3,440
	100	84	1,405
	50	32	335
	10	21	280
	1	14	215
Controls			
Desoxycholate	100	14	165
Buffered saline	---	14	215

[a]Each substance was solubilized in a solution of sodium desoxycholate in buffered saline (3).

phatidylserine in the antithromboplastin and recalcification tests. Similar results were obtained with both the phosphatidyl(distearoloyl)serine and phosphatidyl(stearoloyl, stearoyl)serine (not shown). However, the mixed acid phosphatidylserine was not as active in the Hicks-Pitney test (Table V) as the phosphatide containing two stearoloyl residues.

All the tests employed phosphatidylserines solubilized with the aid of sodium desoxycholate. Similar results were obtained when the synthetic phosphatidylserines were solubilized with albumin solution (3).

Small amounts of dilute alkali were employed to aid in the solubilization with albumin. Phosphatidylserine with protonated carboxyl group is not readily solubilized with albumin solution, whereas sodium or potassium salts of phosphatidylserine are (3).

DISCUSSION

The purpose of this work was to synthesize phosphatides having potent biological activity and fatty acids that would not be autoxidizable. In this paper, we report synthetic phosphatides containing triple bonds or cyclopropane rings rather than double bonds.

The synthesis followed the general scheme devised originally by Baer and Buchena (29) which has been used in our earlier work (30). In this scheme, phosphorus oxychloride is combined with a diglyceride and a protected base, either *t*-butyloxycarbonylaminoethanol or the phthalimidomethyl ester of anisyloxycarbonyl-L-serine. For the removal of protective groups, hydrazine is employed to remove the phthalimidomethyl group as described originally by Nefkens et al. (41). The method of Halpern and Nitecki (32) for removing the *t*-butyloxycarbonyl protecting group was found to be equally applicable to the anisyloxycarbonyl group. This gave an improvement in yield when compared to our previous method employing hydrogen chloride.

The removal of the *t*-butyloxycarbonyl and anisyloxycarbonyl protecting groups by formic acid is essential for the success of the synthesis of cyclopropane phosphatides. Prior to the appearance of the paper of Halpern and Nitecki (32), we had prepared pure phosphatides containing cyclopropane fatty acids by removal of the protecting groups using hydrogen chloride in chloroform. These phosphatides were homogeneous by TLC and paper chromatography. Unfortunately, GLC showed the phosphatidylethanolamine made in this way to contain only 23% of cyclopropane fatty acids with at least 10 other unidentified components. Our products also contained some halogen. It is well known that hydrogen chloride rearranges the cyclopropane ring to methyl olefines (13). Using formic acid to remove protecting groups, it was possible to get phosphatides containing 93% of cyclopropane fatty acids. This is probably as good as can be expected, since the Simmons-Smith reaction is not quantitative (42).

The mixed acid phosphatide, *rac*-phosphatidyl(stearoloyl, stearoyl)serine was hydrolyzed with phospholipase A to determine whether any rearrangement occurred in the synthesis. Since the product of the hydrolysis was stearic acid, no rearrangement in fact occurred. It is well established that this enzyme acts exclusively in the 2-position of 3-phosphoglycerides (43).

One of the major problems in studying the coagulant or anticoagulant activity of phospholipids or considering their use as therapeutic agents is their tendency to autoxidation. The most active of the naturally occurring or synthetic phosphatides with coagulant (3) or anticoagulant (3,4) activity have all contained unsaturated fatty acids. As autoxidation proceeds, their color gradually changes from white to yellow. This is accomplished by a loss of clot accelerating activity of the phosphatidylethanolamines and the appearance of lysophosphatide and other hydrolysis products in the TLC of phosphatidylserine (44). It is hoped that substitution of cyclopropane rings for double bonds will eliminate the problem of autoxidation.

The synthetic phosphatidylserines and phosphatidylethanolamines with triple bonds had the same, strong biological activity as compounds with two double bonds (4,33) or as phosphatidylserine from beef brain in in vitro tests of blood coagulation. The phosphatides containing triple bonds in both fatty acids had more activity (procoagulant for phosphatidylethanolamine and anticoagulant for phosphatidylserine) than those having a triple bond in only one of the fatty acids.

The cyclopropane phosphatidylserine also had as much anticoagulant activity in vitro as beef brain phosphatidylserine. The latter has been shown to be a potent anticoagulant in vivo (1,2). The cyclopropane phosphatidyl ethanolamine had less activity than the PE with triple bonds.

Activity of phosphatides in blood coagulation tests is related to the colloidal state of the phospholipid particles in the aqueous dispersion and particularly to the size and shape of the dispersed particles and their surface charge (3,45-48). It would appear that these character-

istics are similar for the phosphatides containing two triple bonds or two cyclopropane rings to those prevailing with the corresponding naturally occurring phosphatides having strong biological activity. The triple bond and the cyclopropane ring probably resemble the double bond in producing optimal separation of the fatty acid chains in the micelles. Another factor related to solubilization and biological activity may be the lowering of the transition temperature from crystalline to liquid crystalline produced by double bonds in the phospholipid molecule (49,50).

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